Research Article

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Laboratory and field cage studies on female-targeted attract-and-kill bait stations for *Anastrepha suspensa* (Diptera: Tephritidae)†

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Abstract

BACKGROUND: Development of attract-and-kill bait stations for pest fruit flies has been limited by the water solubility of sugar needed as a feeding stimulant and by the volatility of chemical attractants. A wax-based matrix was developed that provides the longevity needed for field use and is biodegradable.

RESULTS: Laboratory bioassays with the Caribbean fruit fly, *Anastrepha suspensa* (Loew), confirmed the efficacy of bait stations containing avermectin, methomyl, spinosad and phloxine B. Field cage studies demonstrated that significant mortality occurred with either 1% (w/v) spinosad or 1% (w/v) methomyl bait stations versus pesticide-free bait stations. Bait stations were exposed to environmental conditions by placing them in trees at the ARS station in Miami, Florida, between tests. There was no loss in efficacy, in spite of exposure to over 360 mm of rainfall over the 56 days of the study, indicating that the bait stations could provide population suppression for at least 1–2 months when used in subtropical environments.

CONCLUSION: A long-lasting, female-targeted fruit fly bait station, such as the one developed herein, could provide a cost-effective option for fruit fly population suppression that would be an important tool in tephritid pest management and control. Additional studies are needed to demonstrate efficacy against wild fruit fly populations and determine deployment strategies. Published 2009 by John Wiley & Sons, Ltd.

Keywords: Caribbean fruit fly; *Anastrepha suspensa*; insecticide; fruit fly management; pest control; bait station

1 INTRODUCTION

There is an increasing need to move from insecticide-based control measures that employ broadcast pesticide application to biologically based control measures that take advantage of cues used by insects to locate food, mates and oviposition sites.[1] Tephritid fruit flies are among the most important pests of fruits and vegetables worldwide. Techniques for tropical tephritid population suppression and eradication include ground and aerial bait spray applications, which are used with other control components in area-wide management approaches.[2] Although many bait spray suppression efforts use naturally occurring biologically based insecticides, there remains public concern regarding adverse effects on the environment and non-target organisms.[3,4] Aerial bait spray is costly and requires a number of applications owing to the short field efficacy of the toxicant. Thus, there is a need for alternatives to the bait sprays currently used. Attract-and-kill bait stations, defined as discrete containers of attractants and toxins, draw the insect to the insecticide rather than relying on broadcast sprays to bring the insecticide to the insect.[5,6]

The first successful development of bait stations for tephritid flies was the combination of the male-targeted lure and feeding stimulant methyl eugenol with an organophosphate insecticide, which formed the basis for the male annihilation technique.[7] This technique has been used successfully against incipient populations of tephritid fruit flies in the *Dacus/Bactrocera* genus in the USA and elsewhere. An ideal bait station for fruit flies should (1) incorporate a female-targeted olfactory cue, a visual cue and a feeding stimulant and toxicant, (2) last at least a month when exposed to adverse environmental conditions and (3) biodegrade naturally.[5,8,9] Sugar is a strong feeding stimulant commonly used in fruit fly bait stations, but there are problems in keeping the sugar available to responding flies. Sugar is highly soluble in water, so exposure to rain will dissolve and remove sugar, which renders the bait station ineffective.[10] The sugar could be protected by placing the material in a rain-fast container,[5,11] but the container would need to be removed from the field unless it was made from biodegradable materials. An alternative is to use a solid wax

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such as paraffin that will not dissolve in water, will incorporate water-soluble materials such as sugar, toxicants and attractants and can be molded into appropriate shapes, and that will provide a mechanism for slow release of the sugar, toxicant and attractant over time to provide needed longevity. Of critical importance is that the final formulation does not repel the fly or inhibit feeding.

This paper reports the development of a wax-based matrix that will attract and kill pest fruit flies, that does not need to be housed in a container and that will stay effective for at least 2 months when exposed to subtropical environments. Studies were conducted with adults of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Previous studies had demonstrated the effectiveness of spinosad against *A. suspensa* in a bait spray[12] or on insecticide-coated spheres,[13] so spinosad was the primary insecticide used in these tests. Other insecticides were evaluated to determine whether the matrix could be used with alternative toxicants. Additional tests evaluated bait station configuration, addition of the food-based attractant ammonium acetate and bait station longevity.

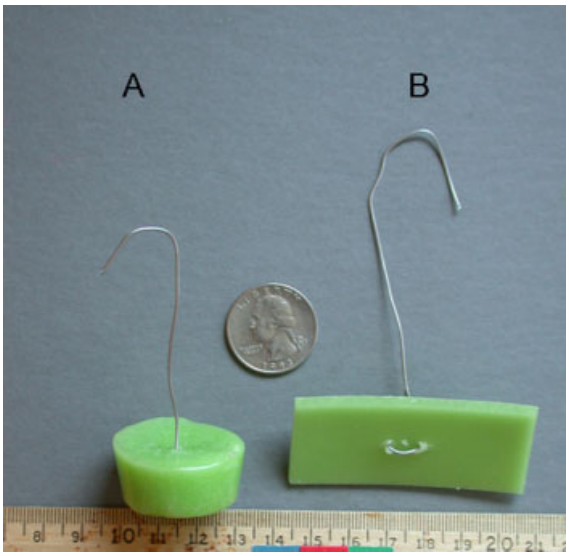

Figure 1. Bait stations prepared using a wax-based matrix and formed into a plug (A) or strip (B). The wire hanger is used to place the bait station in a tree, and the bait stations are green in color to provide a visual cue in addition to the bait station shape. A variety of feeding cues, attractants and insecticides can be added to the bait matrix.

2 MATERIAL AND METHODS

2.1 Matrix

Preliminary studies tested more than 120 different mixtures to develop a formulation that would contain an insecticide, feeding stimulant and attractant, and that would solidify after being poured or molded without the separation of the components. In this paper, 'matrix' will designate a mixture of inert materials that form the bait station. The base matrix, which was used in all tests, was composed of paraffin (GulfWax; Royal Oak Sales, Roswal, GA), a hardener (Elvax-60; Swan Candles, Tacoma, WA) and an emulsifier (Span 60; Uniquema, Wilmington, DE) in a ratio of 16 : 3 : 1 (w/w). Added per 100 mL of base matrix were 24 drops of yellow : green (3 : 1) food coloring (McCormick & Co., Hunt Valley, MD) as a visual cue, and 20 mL of corn syrup (Karo; Bestfoods, Englewoods Cliff, NJ) and 2 g of granulated sugar as feeding stimulants. The paraffin, hardener and emulsifier were combined and were heated with mixing to approximately 80 °C. Additional components were added and mixed for approximately 5 min. Shape is an important visual cue for fruit flies,[14] so bait stations were formed into plugs (2.4 cm diameter by 2.5 cm) to approximate a spherical shape (Fig. 1a) or were cut into strips (2.54 cm by 7.6 cm by 4 mm thickness) and hung horizontally to approximate a leaf shape (Fig. 1b). To form plugs, matrix was poured into clear plastic cup trays (No. 9040; Bio-Serv, Frenchtown, NJ) that had been sprayed with cooking oil (PAM; ConAgra Foods Inc., Omaha, NE). To form strips, matrix was poured into Teflon-lined aluminum sheets that were first sprayed with cooking oil and then had a fabric piece (100% unbleached muslin) placed on the sheet to provide a backing for the strips. To produce plugs, trays were prechilled at 0 °C for 10 min before use and were placed in a freezer for approximately 20 min after adding the matrix plus additives to prevent the mixture from separating before cooling was complete. These steps were not needed when producing strips, as the formulation cooled quickly enough at room temperature to prevent component separation. Once the bait station reached room temperature and solidified, a 15–20 cm piece of galvanized steel wire (24-gauge; Textron, Rockford, IL) was looped through two small holes placed ~6 mm apart in the center of the plug or strip (fabric side up and hung in a horizontal position). The wire was twisted to secure the bait station to the wire, and the long end was then formed into a hook. Bait stations were stored at room temperature until use. Once experiments were initiated, bait stations were hung in non-fruit fly host trees at the Miami ARS station when not being used in bioassays. Bait age was used to indicate number of days the bait stations were exposed to outdoor environmental conditions and subject to weathering.

2.2 Insecticide efficacy bioassays

Flies were obtained as pupae from the colony maintained at the USDA/ARS laboratory in Miami, Florida. Flies were given water and adult food (refined cane sugar + protein hydrolysate, 3 + 1 w/w) and were held in screen cages (30 cm^3). Adult flies were held, and bioassays were conducted in laboratories with a 12 : 12 h light : dark photoperiod at room temperature (24 °C) and ambient relative humidity (36%). Females used in laboratory tests were obtained from mixed-sex cages and ranged in age from 3 to 16 days old. Unless stated otherwise, bioassays were conducted by hanging bait stations individually in cylindrical screen bioassay cages (19.3 cm diameter by 15.3 cm). Ten females were added to each cage, and there were five cages per replicate for a total of 50 flies per treatment per replicate. Bait stations were removed after 4 h, mortality was recorded after 24 h and percentage mortality was used for statistical analyses. Flies were provided with water during the tests.

Tests were conducted in experiment 1 to evaluate the efficacy of various insecticides as the toxicant in the bait station. Bait station plugs were prepared using the following insecticide formulations added to the base matrix at concentrations of 0.25 and 1.0% (w/v) AI: cyromazine 750 g kg^{-1} WP (Trigard; Syngenta Crop Protection, Basel, Switzerland), methomyl 900 g kg^{-1} SP (Lannate; Dupont Ag Products, Wilmington, DE), avermectin 20 g L^{-1} EC (Avid 0.15C; Syngenta Crop Protection), spinosad (spinosyn A and spinosyn D) 116 g L^{-1} SC (Conserve SC; Dow AgroSciences, Indianapolis, IN), suredye (Red Dye 28-Phloxine B; Hilton-Davis, Cincinnati, OH) and dimethoate 230 g L^{-1} EC (Cygon 2E; Southern Agric. Insecticides, Inc., Palmetto, FL). Tests were replicated 10 times.

Trials were conducted in experiment 2 to determine whether bait stations formed into strips would be as effective as those

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that were formed into plugs. Strip formulations would save on amount of matrix per bait station and would be easier to produce. Comparisons were made among four treatments: plugs that contained no insecticide (control), plugs that contained 1% (w/v) spinosad or strips that contained 1% (w/v) spinosad or 1% (w/v) methomyl. Tests were replicated 8 times.

2.3 Addition of ammonium acetate

Ammonium acetate is a well-known attractant for many economically important fruit flies. Laboratory bioassays were conducted in experiment 3 to determine whether the addition of ammonium acetate to the bait station would decrease efficacy by, for example, decreasing/inhibiting fly consumption or degrading the insecticide. For these tests, bait station plugs composed of base matrix and 2% (w/v) spinosad with 0, 1, 2 or 3% (w/v) ammonium acetate (Aldrich Chemical Co., Milwaukee, WI) were tested. To test if time period was a factor in possible insecticide degradation, bait stations that had aged 2, 7, 9, 13, 16, 20, 28 and 33 days in the field were used in the bioassays.

Subsequent field tests were conducted in experiment 4 to determine whether the bait station plugs containing ammonium acetate would attract flies. The standard trapping system for these fruit flies includes MultiLure traps (Better World Manufacturing LLC, Fresno, CA) baited with a two-component food-based synthetic attractant comprised of ammonium acetate and putrescine lures (BioLure; Suterra LLC, Bend, OR).[11] Tests compared capture in MultiLure traps baited with a putrescine lure combined with an ammonium acetate lure or with bait station plugs containing 1% (w/v) spinosad and 1, 2 or 4% (w/v) ammonium acetate. Aqueous propylene glycol [5% (v/v); 300 mL] was added to the base of the traps as the retention agent. The traps were deployed in a block of guava trees at the ARS station in Miami, Florida, and the number of *A. suspensa* captured was recorded every 1–3 days over a 21 day sampling period. Traps were placed, following standard protocol,[15] in five rows of trees with all four treatments in each row, and traps were rotated sequentially within a row when sampled. Distance between traps within a row was ∼20 m, and distance between rows with traps was ∼40 m. There was a row of trees without traps between each row with traps. The numbers of flies per treatment per sample period were summed from capture in all rows, and the number of flies per trap per day per replicate sample period was used for statistical analysis.

2.4 Field cage tests of bait station longevity

Tests were conducted in experiment 5 to determine bait station longevity under field conditions. Bait station strips containing 1% (w/v) ammonium acetate were prepared. Bait stations without insecticide were compared with those with 1% (w/v) methomyl or 1% (w/v) spinosad. For these tests, bioassays were conducted using three screen tents (∼3 m high by 7 m diameter) that were placed on wooden platforms in a shaded area at the Miami ARS station. Two guava plants in pots were placed in each tent. Two bait station strips of the same type were used per field cage, with one placed on each guava plant, and tests compared mortality in bait stations containing no insecticide with that in bait stations containing either methomyl or spinosad. A total of 200 females were released in the morning in each of the tents, and numbers dead and alive were determined after 48 h. When not being used in a bioassay, bait stations were hung in trees and were exposed to outdoor environmental conditions. Tests were initiated when bait stations had aged for 6 days. Subsequent tests were conducted at intervals of 2–8 days for the next 36 days, and the final test was conducted when bait stations had aged 56 days for a total of ten tests (replicates). Average daily temperature (°C) and total daily rainfall (mm) data for this time period in south Florida were obtained from the Florida Automated Weather Network (UF/IFAS, http://fawn.ifas.ufl.edu/data/).

2.5 Statistical analysis

The effect of treatment was determined using Proc GLM.[16] Data were subjected to the Box–Cox procedure,[17] which is a power transformation that regresses log-transformed standard deviations (y) against log-transformed means (x), and data were transformed to stabilize the variance before analysis when necessary. Mean separation was determined using the least significant difference (LSD) test ($P = 0.05$). A one-way ANOVA was used for analysis of data in experiments 1, 2 and 4. A two-way ANOVA using a homogeneity-of-slopes model, with bait age (days) as a regression factor and bait station treatment as a classification factor, was used for analysis of data in experiments 3 and 5.

3 RESULTS

3.1 Insecticide efficacy bioassays

There was no difference in mortality of flies exposed to bait stations without insecticide or with cyromazine (Table 1). Among the remaining insecticides tested at 0.25%, more mortality was obtained with dimethoate than with methomyl, and intermediate mortality was obtained with avermectin, spinosad or suredye. When 1% of the various insecticides were tested, dimethoate, methomyl or spinosad caused the highest mortality and avermectin caused the next highest mortality. Mortality with suredye was still higher than that obtained from the control, but was less than that obtained with avermectin. Interestingly, mortality for suredye or avermectin tended to be lower when tested at 1% than at 0.25%. The higher concentration of these insecticides may have been repellent or have deterred feeding by flies, thus resulting in lower mortality. When the two different bait station configurations were compared in experiment 2 (Table 2), the only effect was due to insecticide

Table 1. Percentage mortality [mean (± SD)] after 24 h of *Anastrepha suspensa* females, 50 per replicate, that were exposed to bait stations with one of several toxicants at two concentrations for 4 h ($n = 10$) in experiment 1[a]

Toxicant	0.25% (w/v) AI	1.0% (w/v) AI
None (control)	5.0 (±7.1) a	5.0 (±7.1) a
Cyromazine	9.0 (±12.0) a	4.0 (±5.2) a
Methomyl	74.8 (±23.0) b	97.8 (±4.6) d
Avermectin	81.4 (±16.8) bc	67.0 (±20.0) c
Suredye	83.0 (±22.1) bc	22.0 (±23.5) b
Spinosad	84.1 (±24.2) bc	96.1 (±6.9) d
Dimethoate	95.1 (±7.0) c	99.0 (±3.2) d
F	47.30	124.10
Df	6, 63	6, 63
P	0.0001	0.0001

[a] Means followed by the same letter within a column are not significantly different (LSD mean separation test, $P = 0.05$).

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Table 2. Percentage mortality [mean (± SD)] after 24 h of *Anastrepha suspensa* females, 50 per replicate, that were exposed to bait stations with different configurations with or without insecticide for 4 h ($n = 8$) in experiment 2[a]

Treatment	Number of flies captured
Bait station plug with no insecticide (control)	8.7 (±10.3) a
Bait station strip with 1% (w/v) spinosad	48.0 (±28.2) b
Bait station strip with 1% (w/v) methomyl	67.4 (±13.4) b
Bait station plug with 1% (w/v) methomyl	69.5 (±29.9) b
F	23.23
df	3, 28
P	<0.0001

[a] Means followed by the same letter are not significantly different [LSD mean separation test of square root ($x + 0.5$) transformed data, $P = 0.05$, means of non-transformed data shown].

Table 3. Number [mean (± SD)] of *Anastrepha suspensa* per trap per day that were captured in MultiLure traps baited with putrescine lures in combination with either bait stations containing ammonium acetate (AA) or with a standard ammonium acetate lure in field tests conducted in Miami, Florida, in experiment 4[a]

Treatment	Number of flies captured
Bait station with 1% (w/v) AA + putrescine lure	0.8 (±1.6) a
Bait station with 2% (w/v) AA + putrescine lure	1.4 (±1.8) ab
Bait station with 4% (w/v) AA + putrescine lure	4.3 (±5.0) bc
AA lure + putrescine lure (control)	8.7 (±10.3) c
F	4.63
df	3, 24
P	0.0108

[a] Means followed by the same letter are not significantly different [LSD mean separation test of log ($x + 1$) transformed data, $P = 0.05$, means of non-transformed data shown].

presence. There was no difference in mortality between plug and strip, or between spinosad and methomyl.

3.2 Addition of ammonium acetate

There was higher mortality (mean ± SD) among flies exposed to any bait station with spinosad (81.9 ± 14.9%) than to the spinosad-free control (5.0 ± 8.9%), regardless of the amount of ammonium acetate added in experiment 3 ($F = 64.65$; df = 3, 24; $P < 0.0001$). There was no effect due to bait station age ($F = 1.19$; df = 1, 24; $P = 0.2854$), and no interaction between bait station age and treatment ($F = 0.78$; df = 3, 24; $P = 0.5155$) (data not shown). Thus, there was no loss in efficacy over the 33 days of the test.

Field tests conducted in experiment 4 found that the greatest numbers of *A. suspensa* adults were captured in traps baited with either the ammonium acetate lure or with the 4% ammonium acetate bait station in combination with the putrescine lure (Table 3). There was a direct relationship between decrease in concentration of ammonium acetate in the bait station and decrease in capture, and traps baited with the 1% ammonium acetate bait stations and putrescine captured ∼9% of the flies obtained in synthetic lure-baited traps.

3.3 Field cage tests of bait station longevity

The number of flies alive at the end of the trial was used for analysis in experiment 5 because few dead flies were recovered from the field cages as a result of predators (mainly ants and spiders) getting inside the field cages and removing dead flies. Temperature and rainfall over the course of the study as well as dates of the bioassays are shown in Fig. 2. Approximately 368 mm of rainfall occurred between the start and the end of the study. However, there was no effect of days weathered (bait age; $F = 0.95$; df = 1, 24; $P = 0.3391$), nor was there an interaction between bait age and treatment ($F = 1.79$; df = 2, 24; $P = 0.1892$). Thus, there was no loss in efficacy over the 56 days of the test period (data not shown). There was, however, an effect due to treatment (Table 4). The highest survival was in tests with bait stations without insecticide, intermediate survival was in tests with spinosad bait stations and the lowest survival was in tests with methomyl bait stations.

4 DISCUSSION

The wax-based matrix used in these studies allows incorporation of various additives, including insecticides, feeding stimulants

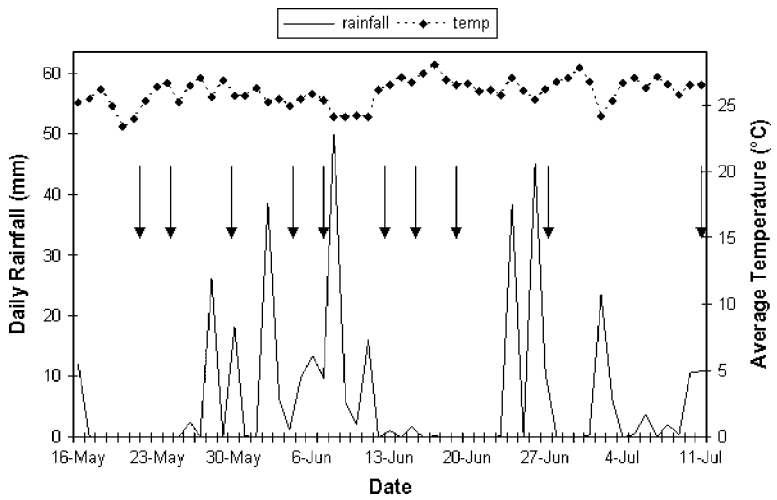

Figure 2. Environmental conditions including average temperature (°C, dashed line and diamond) and total daily rainfall (mm, solid line) to which bait stations were exposed during tests conducted in Miami, Florida, in experiment 5. Arrows indicate dates of laboratory bioassays of bait station efficacy.

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Table 4. Number [mean (± SD)] of *Anastrepha suspensa* that were alive after exposed to bait stations with or without insecticide for 48 h in experiment 5. 200 females were released per test ($n = 10$)[a]

Treatment	Number of flies alive
Bait station strip with 1% (w/v) methomyl	1.9 (±4.7) a
Bait station strip with 1% (w/v) spinosad	9.4 (±5.1) b
Bait station strip with no insecticide (control)	72.5 (±22.3) c
F	89.89
df	2, 27
P	<0.0001

[a] Means followed by the same letter are not significantly different [LSD mean separation test of log ($x + 1$) transformed data, $P = 0.05$, means of non-transformed data shown].

and attractants. During the initial stages of this research it was determined that, in addition to paraffin, a hardener would be needed to provide mechanical stability, and an emulsifier to ensure that the materials did not separate while cooling. The corn syrup was an important additive. Without syrup, many of the formulations became brittle and lost the green color within 2 weeks of placement in the field (Heath RR and Schnell E, unpublished data). It was found that the efficacy of the bait station was reduced immediately after exposure to a rainfall event but was restored within 24 h (Heath RR and Midgarden D, unpublished data). This was hypothesized to be due to movement of additional feeding stimulant back to the surface of the station to replace material that had been washed off by the rain. Thus, the bait stations in the present studies remained effective for more than 50 days, even when exposed to subtropical rain conditions. Because the nature of the transport mechanism is critical to bait station performance, further investigation of the mechanism is ongoing. The fabric covering the top of the bait station strips served several purposes. It helped strips to retain a horizontal leaf-mimic shape, as those without fabric sagged and tended towards an inverted U-shape. It provided a better support for the wires used for hanging, functioned as a roof and provided some additional protection from the weather.

Additional tests are needed to determine the longevity of release of attractant chemicals from the bait stations. The green color and the shape of both plugs and strips are highly effective visual cues, which have been shown to be strong attractants for tephritid fruit flies even without an olfactory lure.[18] Slow-release mechanisms are needed for many insect attractants because of their high volatility.[6] Studies in experiment 3 determined that the addition of ammonium acetate did not deter fruit fly feeding on the bait stations and did not degrade the spinosad, but this experiment did not address longevity of chemical release. Ammonium acetate is highly volatile, and results from experiment 4 suggest that volatile chemicals were still being released from bait station plugs after 3 weeks. Unbaited traps or traps without ammonia typically capture 0% or <5%, respectively, of *Anastrepha* spp. in comparison with synthetic lure-baited traps.[19,20] Thus, the release of ammonium acetate from the bait station even at the lowest concentration was enough to attract flies to the traps. Additional studies are needed to determine the longevity of ammonia release from the bait station strips, and development of new analytical techniques may allow quantification of ammonia release directly.[21]

Population suppression using bait stations is a less labor-intensive version of population suppression using female-targeted sticky and McPhail-type traps. Sticky spheres placed around the periphery of an orchard have been used for control of the apple maggot, *Rhagoletis pomonella* (Walsh).[22] Mass trapping using synthetic lure-baited McPhail-type traps has been demonstrated for control of *C. capitata* in large-scale field tests conducted in Spain.[23] Sticky traps require frequent maintenance, as the sticky material becomes deactivated by build-up of insects captured or by dust/debris.[8] Similarly, McPhail-type traps need to be emptied periodically, and often the liquid in the base needs to be replenished for the trap to remain active. Bait stations developed for fruit flies include sugar/flour spheres (7–9 cm diameter) coated with latex paint containing various insecticides.[10,15,24–27] However, problems including fungal growth and consumption by rodents have discouraged the field use of these spheres.[27,28] An alternative design used wooden or plastic pesticide-painted spheres, topped with a sugar/paraffin piece protected by a wire mesh guard;[13,29,30] however, high cost has limited its development (Liburd OE, private communication). Another type of tephritid bait station was developed by Mangan and Moreno.[5] This uses a liquid protein bait formulation developed for tephritid bait spray application.[31] These bait stations use a housing to contain the bait and to protect the bait from the environment.

Wax-based bait stations could provide a cost-effective option for fruit fly population suppression. Materials for the base matrix are inexpensive, are available in bulk quantities and would cost less than five cents per bait station plus the cost of the insecticide. The insecticide is the most expensive component, but a variety of toxicants could be used, and the total pesticide amount is much less than that used in weekly bait spray application. By using alternative material for the hook, the wax-based bait station would provide an inexpensive, biodegradable option that could be left in the field to decay naturally. Alternatively, for use in high-value crops when the grower wants higher efficacy and can retrieve the units from the field at the end of the season, the wax-based bait station could be combined with synthetic lures, which would provide long-lasting controlled release of fruit fly attractants.

The matrix developed in this study could be used for insects other than tephritids by the addition of other pesticides or feeding cues. For example, bait stations using an appropriate feeding cue were effective against several species of stored-product beetles in laboratory trials (Heath RR and Epsky ND, unpublished data). Bait stations will exert continuous pressure on the pest insects, as opposed to repeated short-duration pressure from bait spray application. Field trials conducted in Argentina in an area with a low fruit fly population level demonstrated that population suppression, as indicated by either adult capture in traps or fruit infestation level, using the bait station strips with methomyl that have been described in this paper was comparable with that obtained with bait spray application.[32] Additional research is needed to test these bait stations for other tephritid species, under different environmental conditions, and to determine deployment factors such as the number of bait stations per hectare and the effectiveness of bait stations versus mass trapping approaches or bait spray application. These and a myriad of questions will need to be researched before the full optimization for population suppression using bait stations is realized.

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